NGS sample preparation:

1. Extract DNA, as usual, following the protocol. Store samples at +4^{0}C
2. Run PCR: 3 reactions per 1 sample, following the protocol. Store samples at -20^{0}C
3. Purify PCR products:
    - Take a new PCR strip
    - Transfer 10ul from each PCR product to a new tube (3 tubes total)
    - Add 4ul of ExoSAP-IT to each tube
    - Run protocol '80' in the PCR machine
4. Prepare a sample for sequencing:
    - After purification is done, take the samples out and combine them in one new 0.5ml- tube (42ul)
    - Transfer 30ul to a new labeled 0.5ml-tube -> this is the tube which will be sent for sequencing
    - Use the leftovers (12ul) to run a gel, to verify the DNA presence before submission

Sample submission:

1. Create account in GENEWIZ
2. From your account open a new order -> NGS->amplicon sequencing->AmpliconEZ
3. Fill out the form following the steps in the submission form
4. Print out the order confirmation
5. Place the order confirmation and the sample (0.5ml- tube) in a ziplock bag and store at -20^{0}C until submitted
6. Before 1pm on the day of submission, place the bag in GENEWIZ dropbox – 3rd floor, on the floor near room 3159 (across the stairs)
7. You will receive the follow-up emails from GENEWIZ. The sequences will be ready in ~8 business days. To retrieve the results, follow the instructions in the last email from GENEWIZ (the results are not available in your GENEWIZ account).

For submission form:

Step 1: Basic Information

- Sample Buffer:Water
- Size of Amplicon (bp):390

Step 2: Concentration

- Method of DNA Measurement:Qubit
- Normalized to 20 ng/µL?:Yes
- DNA Concentration (ng/µL):20
- Sample Volume (µL):30
- Total DNA Amount (ng):600
- Gel Image?:No

Step 3: Data Analysis

- Number of Targets:One
- Reference Sequence:
- Forward Primer Sequence:cgaaatcggtagacgctacg
- Reverse Primer Sequence:ggggatagagggacttgaac
- Need Analysis?:Yes
- Available Analysis:Unique sequence identification and abundance analysis

*Comments:
- we do not have a reference sequence
- we do not submit adapters (just primers) -> $75/sample
- in the example above the primer sequences are for trnl-gene.
- here are the sequences for rbcl-gene (the one you will use, check the tube labels for the sequence):

rbcLa-F, fwd_seq:
atgtcaccacaaacagagactaaagc

rbcLa-R, rev_seq:
gtaaaatcaagtccaccrcg